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Short communication

Using a gestalt to measure the quality of in vitro responses

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Abstract

Overall ''quality'' of in vitro responses can sometimes be difficult to assess using measured response variables (e.g., shoot number and height, and callus fresh weight). Gestalt Theory is the idea that the whole is perceived to be greater than the sum of the individual parts. To determine if a gestalt assessment could be used to assess quality of in vitro responses two plant tissue culture systems were examined—*Brugmansia x candida* shoot multiplication and sweet orange nonembryogenic callus growth. The gestalt assessment of each system was compared to measured responses—shoot number, leaf length and width for *Brugmansia x candida*, and fresh weight accumulation for citrus. The gestalt analysis modeled as precisely as the measured response variables for both in vitro systems while satisfying the statistical assumptions necessary for a valid analysis. We concluded that the gestalt response is a valid response as it was indistinguishable, in terms of statistical quality, from the measured responses.

Keywords: Citrus; Sweet orange; *Brugmansia x candida*; Response surface methodology

1. Introduction

Experimental research typically involves designing experiments that are composed of factors to vary and responses to measure. When the factors and responses are well chosen the result is a clear answer to the question the experiment was designed to answer and, some new understanding of ''what does what to what'' (Box et al., 2005). Hence, selecting the factor types, numbers, and ranges and how the responses will be measured become the most important components of the experiment. Measuring responses is just that, a precise measurement that may include weights, counts, rankings, numerical values, ratios, and/or various combinations of these measures. Examples of some commonly measured responses in plant tissue culture experimentation include fresh and dry weight accumulation, numbers and sizes of plant organs and structures (e.g., roots, shoots, leaves, and somatic embryos), growth rates, enzyme activity, and secondary metabolite, mineral ion, and gene transcription levels.

However, overall ''quality'' may, in some cases, be difficult to estimate using these types of metrics. The reason relates to Gestalt Theory where the overall whole is perceived to be greater than the sum of the individual parts (Palmer, 1999). This approach is commonly used in food science for measuring consumer food preferences using sensory evaluations. Significant improvements in food products and processes have been achieved by varying input factors to optimize overall consumer acceptability (Koeferli et al., 1998). The physical and chemical properties of a food are easily measured in a laboratory; however, consumer preference is more complicated as it includes not only differences between individuals but is an overall quality measure generally based on some form of the hedonic scale (Peryam and Pilgrim, 1957). Some examples include the ''overall acceptability'' of frankfurters where the pork fat was replaced with olive oil, pectin, and salt (Pappa et al., 2000); the aroma, texture, flavor, feeling, and spreadability of various chocolate peanut butter formulations and roasts (Chu and Resurreccion, 2005); the ''overall degree of liking'' of vanilla ice cream with varying levels of sugar and fat (Guinard et al., 1996); or the ''overall acceptability'' of tropical jam composed of varying proportions of pineapple, carambola, and papaya (Abdullah and Cheng, 2001).

Can the overall quality of an in vitro response be quantified, analyzed, and improved by using the ''gestalt'' as a response? Using the American Society for Quality Control (http://www.asq.org/index.html) definition of quality as, ''the characteristics of a product or service that bear on its ability to satisfy stated or implied needs,'' we present preliminary

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evidence, using two different plant tissue culture systems, that complex, in vitro characteristics such as overall quality can be directly measured and analyzed with precision.

2. Materials and methods

2.1. Plant material and tissue

Shoot cultures were initiated from *Brugmansia x candida* 'Creamsickle' (herein referred to as *Brugmansia*) from greenhouse grown mother stock plants grown with standard horticultural practices. Cultures were initiated from lateral buds and shoot tips dissected to about 5–10 mm tall followed by a disinfestation protocol that began with 95% ethanol for 1 min with agitation. After decanting the excess ethanol the explants were placed into a solution of 0.525% sodium hypochlorite plus two drops of Tween-20 per 100 ml with agitation. After 15 min the sodium hypochlorite solution was decanted and the explants were rinsed with sterile water. The lateral buds and shoot tips were further dissected to about 3–6 mm tall before explanting onto the culture initiation medium. The initiation medium was MS basal medium (Murashige and Skoog, 1962), 3% (w/v) sucrose, myo-inositol 100 mg/l, thiamine HCl 1 mg/l, pyridoxine HCl 1 mg/l, nicotinic acid 1 mg/l, glycine 2 mg/l, 6-benzylaminopurine (BA) 1.1 μM, pH 5.7, and bacteriological agar (USB Corporation, Cleveland, Ohio, USA) 9.0 g/l. The medium was autoclaved in 500 ml volumes for 20 min at 121 °C and then poured into previously autoclaved 25 mm × 100 mm flat-bottomed glass culture tubes with polypropylene caps (Magenta Corporation, Chicago, Illinois, USA) at 15 ml of medium per tube. The culture tubes containing the liquid medium were cooled in a vertical position in polypropylene racks (Magenta Corporation). Four weeks after initiation the cultures were transferred to the same medium in polypropylene capped tubes except that the BA concentration was reduced to 0.5 μM. There after, the multiplying clumps of precociously enhanced lateral buds were cultured onto the same medium in 77 mm × 77 mm × 77 mm polycarbonate vessels with polypropylene closures (Magenta Corporation) at 35 ml medium per vessel. The cultures were grown in a growth room under low light (26 μmol m^{-2} s^{-1}) provided by cool-white fluorescent lamps, constant 27 °C, and a 16-h photoperiod.

The callus culture was a four year old nonembryogenic cell line (Val01) developed from epicotyl explants of in vitro grown seedlings of *Citrus sinensis* (L.) Osbeck cv. 'Valencia.' Seed were germinated in MS basal medium (Murashige and Skoog, 1962) with 3% (w/v) sucrose and without plant growth regulators. One centimeter epicotyl explants were excised from 15–21 days old seedlings and placed onto MT medium (Murashige and Tucker, 1962) with 1 μM 2,4-dichlorophenoxyacetic acid (2,4-D), 1 μM 6-benzylaminopurine (BA) and 100 mg l^{-1} casein hydrolysate. The cultures were grown in a growth cabinet under low light (15–20 μmol m^{-2} s^{-1}), provided by cool-white fluorescent lamps, constant 27 °C, and a 4-h photoperiod. After 6 months of selection, a rapidly growing callus designated Val01 was obtained. For maintenance, the callus was grown on the same medium.

2.2. Experimental approach

Brugmansia shoot multiplication and sweet orange callus growth were used to test the use of a gestalt-type response as a quality measure in comparison to measured responses. For *Brugmansia* shoot multiplication the objective was to determine the "optimal" level of BA. The experimental medium was the same as above except with varying (0–2.2 μM) concentrations of BA. The BA concentration range was identified from a preliminary screening experiment that sampled the 0–5 μM range. The [BA] treatments included 0, 0.37, 0.73, 1.10, 1.47, 1.83, and 2.20 μM and were selected for estimating a single factor response surface modeled by a cubic polynomial. The experimental vessel was a 25 mm × 100 mm flat-bottomed glass tube with a polypropylene cap and 15 ml of medium. Five mm long shoot tips from the multiplying clump cultures described above were cultured onto the experimental formulations with a 4 week subculture. Following the first subculture, the basal clump was recultured onto the same experimental medium in separate culture tubes for an additional 4 weeks to reduce carryover effects. Six pseudo-replicate 25 mm × 100 mm tubes were used per treatment. Three true replicates (i.e., six pseudo-replicates per replicate) were used to provide an estimate of pure error. Data were collected at the end of the second 4 week subculture period.

Four response variables were selected for measurement—shoot number, third leaf length and width, and overall quality (the "gestalt"). Shoot number was determined by counting the number of shoots >5 mm. Leaf measurements were conducted on the third leaf of the dominant shoot. Quality was determined by an overall visual assessment of each culture tube; a "healthy" and "vigorous" culture relative to the other BA levels was scored as a "3," a "poorly" growing culture was scored as a "1," and a culture that was not clearly a "3" or a "1" was scored a "2."

For the sweet orange callus cultures the objective was to regulate the biomass accumulation by varying the basal salt composition. To minimize the number of treatments the MS basal salts were divided into three categories (factors), macro ions, micro ions, and Fe; each factor's range was set as a multiple of the basal MS salt level (Table 1). The experimental design was a D-optimal response surface modeled by a quadratic polynomial. The design was modified to include additional points for lack-of-fit and pure error estimation. The design included 10 model and 16 residual (11 lack-of-fit and 5

Table 1
Factors and their ranges for the callus culture basal salt experiment

Factors		Salts	Range
Factor A	Macro ions	NH_4NO_3, KNO_3, $CaCl_2{\cdot}2H_2O$, KH_2PO_4, $MgSO_4{\cdot}7H_2O$	0.1×–1×
Factor B	Micro ions	$MnSO_4{\cdot}H_2O$, $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}7H_2O$, KI, $CoCl_2{\cdot}6H_2O$, H_3BO_3, $Na_2MoO_4{\cdot}2H_2O$,	0.3×–3×
Factor C	Fe	$FeSO_4{\cdot}7H_2O$ chelated with $Na_2EDTA{\cdot}2H_2O$	0.3×–3×

Ranges are multiples of the basal MS level.

pure error) design points. Five grams of callus were subcultured onto each treatment formulation for acclimation to the formulation, then subcultured again (1 g) onto each treatment formulation. Six pseudo-replicate 100 mm × 15 mm culture dishes were used per treatment. Five true replicates were used to provide an estimate of pure error; this provided a >95% chance of detecting an effect of two standard deviations for the main effects and >90% for the two-way interactions and quadratic terms.

Two response variables were selected for measurement on day 15—fresh weight accumulation and overall quality. Fresh weight accumulation was measured by the proportional increase in accumulated fresh weight by day 15. Overall quality was determined by a visual assessment (i.e., gestalt) of each culture; a ''healthy'' culture were scored as a ''3,'' a ''poorly'' growing culture was scored as a ''1,'' and a callus that was not clearly a ''3'' or a ''1'' was scored a ''2.''

2.3. Design analysis

The ANOVA data were the means of the six culture vessels (i.e., pseudo-replicates) that comprised each treatment. Pure error was estimated by replicating three and five treatment points in the *Brugmansia* and sweet orange callus experiments. The treatment points were selected algorithmically by the software to adequately sample the experimental design space. For each experiment the highest order polynomial model where additional model terms were significant at the 0.05 level was analyzed by ANOVA. Model adequacy tests as described by Anderson and Whitcomb (2005) and calculated by Design Expert® 7 (Stat-Ease, Inc., Minneapolis, MN) were as follows:

1. Normality assumption—a normal probability plot of the internally studentized residuals was examined; the assumption is satisfied if the residuals plot closely along a line.
2. Constant variance assumption—a plot of the internally studentized residuals versus predicted response value was examined; the assumption is satisfied if the points fall within the interval of −3 to +3 standard deviations (i.e., sigma), exhibit random scatter, and do not show a ''megaphone'' (>) pattern where the residuals increase with the predicted response.
3. Outlier *t*-values—a statistic calculated for each point; a point outside ±3.5 standard deviations is defined as an outlier and indicate either a problem with that point, or with the chosen model.
4. Box–Cox plot—used to determine if the data require a power law transformation. A transformation is recommended, based on the best lambda value, which is found at the minimum point of the curve generated by the natural log of the sum of squares of the residuals (Box and Cox, 1964; Myers and Montgomery, 2002; Anderson and Whitcomb, 2005).
5. Lack-of-fit (LOF) test—additional design points were included in every experiment for this test. A significant LOF indicates the model may not be capturing all the signal in the observations.
6. Predicted versus actual values plot—points that are randomly scattered along and around a 45° line (i.e., perfect correlation) indicate the model appears to be unbiased when predicting new observations.
7. R^2, adjusted-R^2, and predicted-R^2—calculated as follows: $R^2 = 1 - SS_{residuals}/(SS_{model} + SS_{residuals})$ and is the fraction of overall variation explained by the model; adjusted-$R^2 = R^2$ adjusted for the number of terms in the model relative to the number of points in the design; predicted $R^2 = 1 - (PRESS/SS_{Total})$ where PRESS is the ''predicted residual sum of squares'' and ''measures how well the model is likely to predict the response in a new experiment'' (Montgomery, 2005).
8. Adequate precision—a measure of signal to noise in the data; it compares the predicted values at the design points to the average prediction error. Ratios greater than 4 are preferred (Anderson and Whitcomb, 2005).

The software application Design-Expert® 7 (Stat-Ease, Inc., Minneapolis, MN) was used for experimental design construction, model evaluation, and all analyses.

3. Results

3.1. Brugmansia shoot multiplication—shoot number

Numbers of shoots >5 mm produced over a [BA] range of 0–2.2 μM is shown in Fig. 1a. The cubic polynomial that describes shoot number is as follows: shoot # = 0.65486 + 9.73087*[BA] − 9.00116*[BA]^2 + 2.07420*[B-2.07420*[BA]^3. No deviation from normality was detected as the points clustered closely to the line (Fig. 1b). The variance appeared constant as the points all fell within the three sigma limits and the scatter does not reveal any obvious distortions (Fig. 1c). The outlier *t*-test did not reveal any data points that could be considered as outliers and therefore suspect (plot not shown). Examination of the Box–Cox plot (plot not shown) did not indicate a need for data transformation. The lack-of-fit test was not significant ($p = 0.5412$) indicating no additional variation in the residuals could be removed with a better model. The predicted shoot number versus the actual shoot number plot (Fig. 1d) and the predicted R^2 of 0.76 indicated that the model is useful for prediction.

A summary of the ANOVA, R^2, and adequate precision statistics for shoot # is presented in Table 2. The overall cubic model was highly significant and included three highly significant terms—the linear, quadratic, and cubic terms of [BA]. ''Adequate precision'' measured 11.26. The model predicts that the highest shoot number, 3.77, should occur at 0.72 μM BA. The adjusted-R^2 statistic indicated that 88% of the variation in observed shoot number was explained by the cubic model. More importantly, the predicted R^2 indicates that 75% of the variation in predicting shoot number is captured by this model.

3.2. Brugmansia shoot multiplication—length and width of third leaf

The two response variables length and width of the third leaf of the dominant shoot are shown in Fig. 2a and b. The quadratic

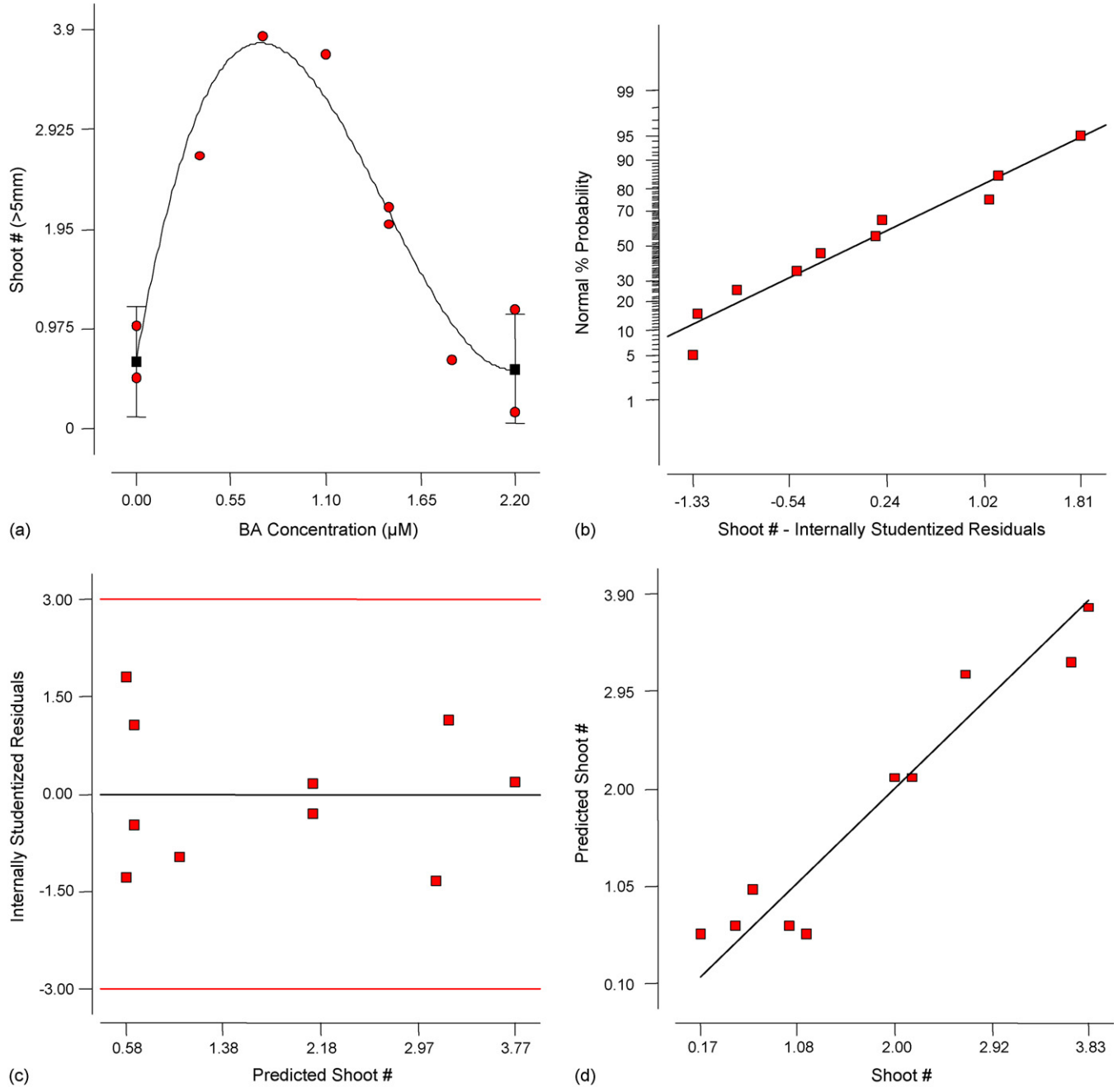

Fig. 1. Effect of BA concentration on *Brugmansia* shoot number, including diagnostic plots of model adequacy. (a) Shoot number vs. [BA]. Shoot number is the number of shoots >5 mm by day 28. The plotted cubic polynomial equation is shoot # = 0.65486 + 9.73087*[BA] − 9.00116*$[BA]^2$ + 2.07420*$[BA]^3$. (b) Normal plot of residuals for internally studentized residual shoot number data. (c) Residuals vs. predicted shoot number plot. (d) Predicted vs. actual shoot number plot.

and cubic polynomials that describe leaf length and width are as follows: third leaf length (mm) = 13.77815 − 9.62391*[BA] + 2.37266*$[BA]^2$; and, third leaf width (mm) = 5.68541 + 11.83316*[BA] − 16.02287*$[BA]^2$ + 4.62764*$[BA]^3$. The measures of model adequacy described above for shoot # were examined for third leaf length and width and it was determined that both models are highly significant and are adequate predictors of these two responses (Table 2).

The quadratic model for third leaf length predicts that leaf length decreases as BA concentration increases, with the longest leaves, 13.77 mm, predicted to occur at 0 μM BA and the shortest leaves, 4.08 mm, predicted to occur at 2.2 μM BA. The cubic model for third leaf width predicts that the widest leaves, 8.18 mm, should occur at 0.45 μM BA and, the narrowest leaves, 2.03 μM, should occur at 1.85 μM BA. The predicted R^2-values for third leaf length and width are 0.8367 and 0.9185 and indicate that both models are suitable for predicting new observations.

3.3. *Brugmansia* shoot multiplication—gestalt

The gestalt response plotted over the BA concentration range is shown in Fig. 3a. The gestalt was not a measured

Table 2
ANOVA for Brugmansia response variables shoot #, leaf length and width, and the gestalt

Term	Shoot #[a]	Leaf length[b]	Leaf width[c]	Gestalt[d]
Overall model—*F*-value	23.38	51.55	72.58	50.78
(*p*-Value)[*]	(0.0010)	(<0.0001)	(<0.0001)	(0.0001)
Lack-of-fit—*F*-value	0.88	0.33	0.16	0.099
(*p*-Value)[**]	(0.5412)	(0.8430)	(0.9200)	(0.9554)
R^2	0.9212	0.9364	0.9732	0.9621
Adjusted R^2	0.8818	0.9183	0.9598	0.9432
Predicted R^2	0.7553	0.8367	0.9185	0.8927
Adequate precision[***]	11.26	15.174	23.193	18.268

[a] The *F*-value (*p*-value) of the individual shoot # cubic model terms were [BA] − 19.76 (0.0044); $[BA]^2$ − 57.09 (0.0003); $[BA]^3$ − 15.48 (0.0077).
[b] The *F*-value (*p*-value) of the individual leaf length quadratic model terms were [BA] − 89.76 (<0.0001); $[BA]^2$ − 10.32 (0.0148).
[c] The *F*-value (*p*-value) of the individual leaf width cubic model terms were [BA] − 156.99 (<0.0001); $[BA]^2$ − 8.13 (0.0291); $[BA]^3$ − 90.32 (<0.0001).
[d] The *F*-value (*p*-value) of the individual gestalt cubic model terms were [BA] − 63.80 (0.0002); $[BA]^2$ − 105.93 (<0.0001); $[BA]^3$ − 62.89 (0.0002).
[*] The *F*-value for the overall model and the probability of a larger *F*-value. A $p < 0.05$ indicates a significant effect on the response measured.
[**] A $p > 0.05$ indicates no additional variation that might be accounted for using a better model.
[***] Design-Expert recommends a value greater than 4 to ensure adequate predictions.

response but rather an overall assessment. The scoring method was simple. A culture was evaluated as clearly acceptable (scored a 3), clearly not acceptable (scored a 1), or a third category (scored a 2) reserved for cultures that did not fit clearly acceptable or not acceptable. Acceptability was based on a relative measure within the range of responses observed. For example, a culture given a score of 3 did not mean that there was no room for improvement, but rather that this culture was, relative to the other cultures, as good as was observed among all the cultures. This made scoring rapid and considerably faster than the quantitative measurements. No deviation from normality was detected as the points clustered closely to the line (Fig. 3b). There is some slight curvature that can be removed with any of several data transformations (e.g., log) but the curvature is subtle and the resulting model diagnostics are essentially unchanged (analysis not shown). The variance appears relatively constant as the points all fall within the three sigma limits and the scatter does not reveal any obvious distortions (Fig. 3c). The outlier *t*-test and Box–Cox plot (not shown) did not indicate any outliers or a need for data transformation. The lack-of-fit was not significant ($p = 0.9554$). The predicted gestalt versus the actual gestalt plot (Fig. 3d) indicated that the model is useful for prediction as the points are randomly scattered around the 1:1 regression line.

A summary of the ANOVA, R^2, and adequate precision statistics for the gestalt response is presented in Table 2. The cubic model is highly significant. The cubic model for the gestalt predicts that the ''best'' overall shoot multiplication occurs at 0.69 μM BA. The predicted R^2-value for the gestalt response is 0.8927 and indicates that the model is suitable for predicting new observations.

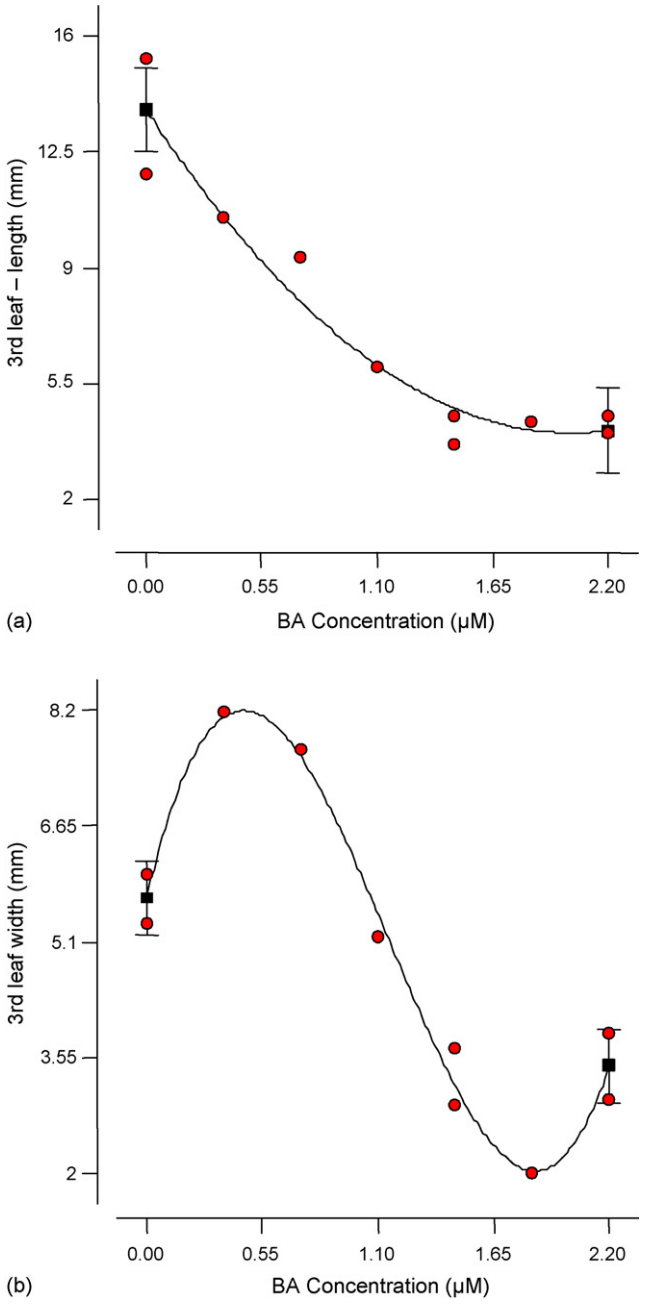

Fig. 2. Effect of BA concentration on *Brugmansia* third leaf length and width of the dominant shoot. (a) The plotted quadratic polynomial equation is the length (mm) = 13.77815 − 9.62391*[BA] + 2.37266*$[BA]^2$ + 1.33357*$[BA]^3$. ^{3}. (b) The plotted cubic polynomial equation is width (mm) = 5.68541 + 11.83316*[BA] − 16.02287*$[BA]^2$ + 4.62764*$[BA]^3$.

3.4. Sweet orange callus growth—fresh weight accumulation

Fresh weight accumulation was the proportional increase in fresh weight of sweet orange callus and is shown in Fig. 4a where the micronutrient dimension is set at 3× (note that this dimension is not plotted on this figure), the level where fresh weight

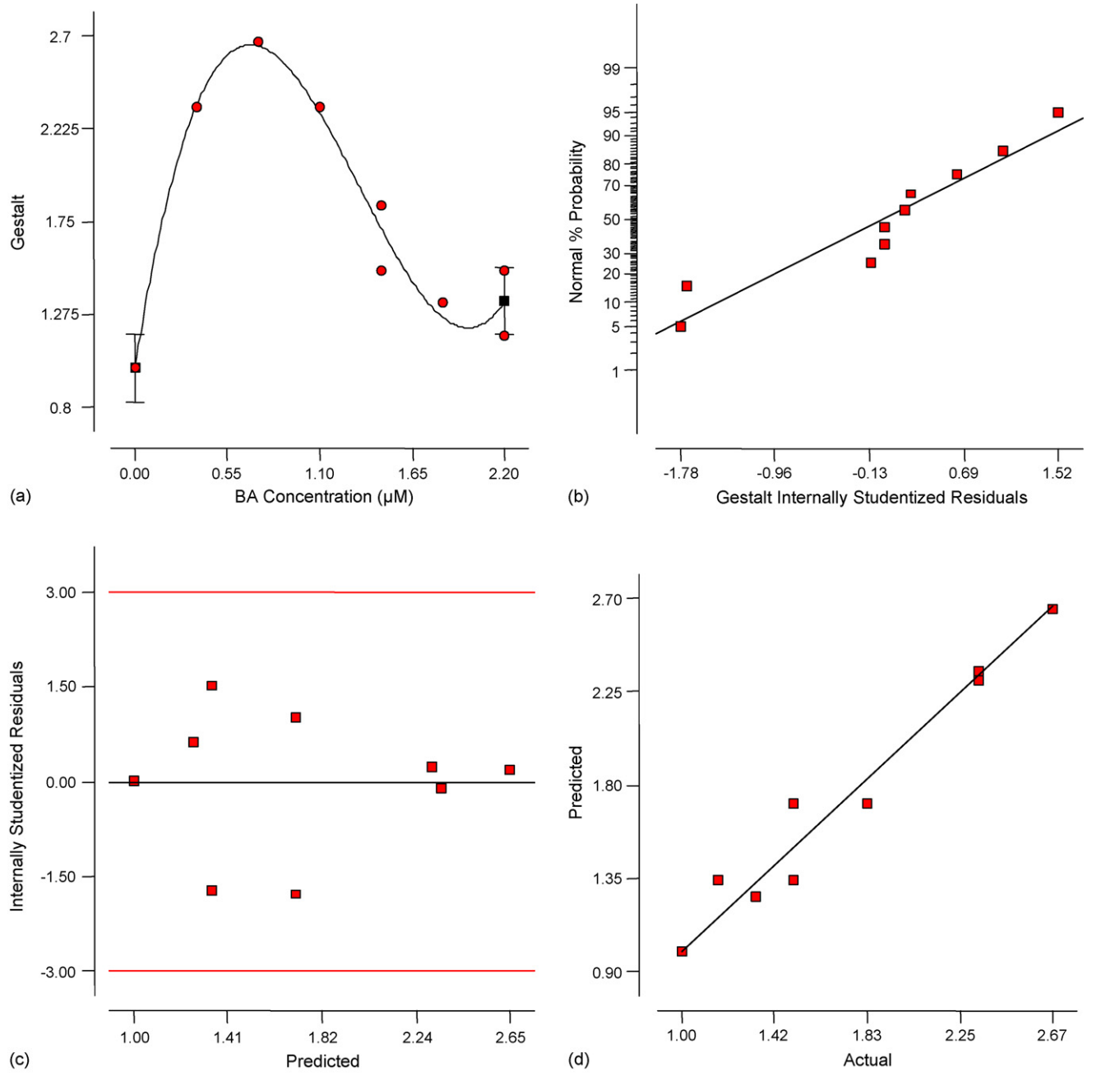

Fig. 3. Effect of BA concentration on *Brugmansia* gestalt including diagnostic plots of model adequacy. (a) Gestalt vs. [BA]. The plotted cubic polynomial equation is the gestalt = 0.99953 + 5.44482*[BA] − 5.33767*$[BA]^2$ + 1.33357*$[BA]^3$. (b) Normal plot of residuals for internally studentized residual gestalt data. (c) Residuals vs. predicted gestalt plot. (d) Predicted vs. actual gestalt plot.

accumulation was highest. The data were first log transformed based on analysis of the Box–Cox plot. The quadratic polynomial that describes fresh weight growth is as follows: $\log_{10}$ fresh weight growth = −0.50618 + 0.030607(Macros) − 0.042616 (Micros) + 0.36569(Iron) + 0.39058(Macros)(Iron) + 0.097613 (Micro)(Iron) − 0.20759$(Iron)^2$. The model adequacy plots, as discussed above, show that the quadratic model adequately describes the $\log_{10}$ response and can be used to predict new observations (Figs. 4b–d).

A summary of the ANOVA, R^2, and adequate precision statistics for fresh weight accumulation is presented in Table 3. The overall quadratic model was highly significant and included five highly significant terms—the linear main effects of Macros and Micros, the interaction effects of Macros*Iron and Micros*Iron, and the quadratic main effect of $Iron^2$. ‘‘Adequate precision’’ measured 14.926 thereby indicating that the model is adequate for prediction (Anderson and Whitcomb, 2005). The adjusted-R^2 statistic indicates that 75% of the variance in observed fresh weight accumulation was explained by the model. The predicted R^2, a more useful statistic, indicates that 65% of the variance in predicting shoot number would be captured. We conducted a simple test of the model by

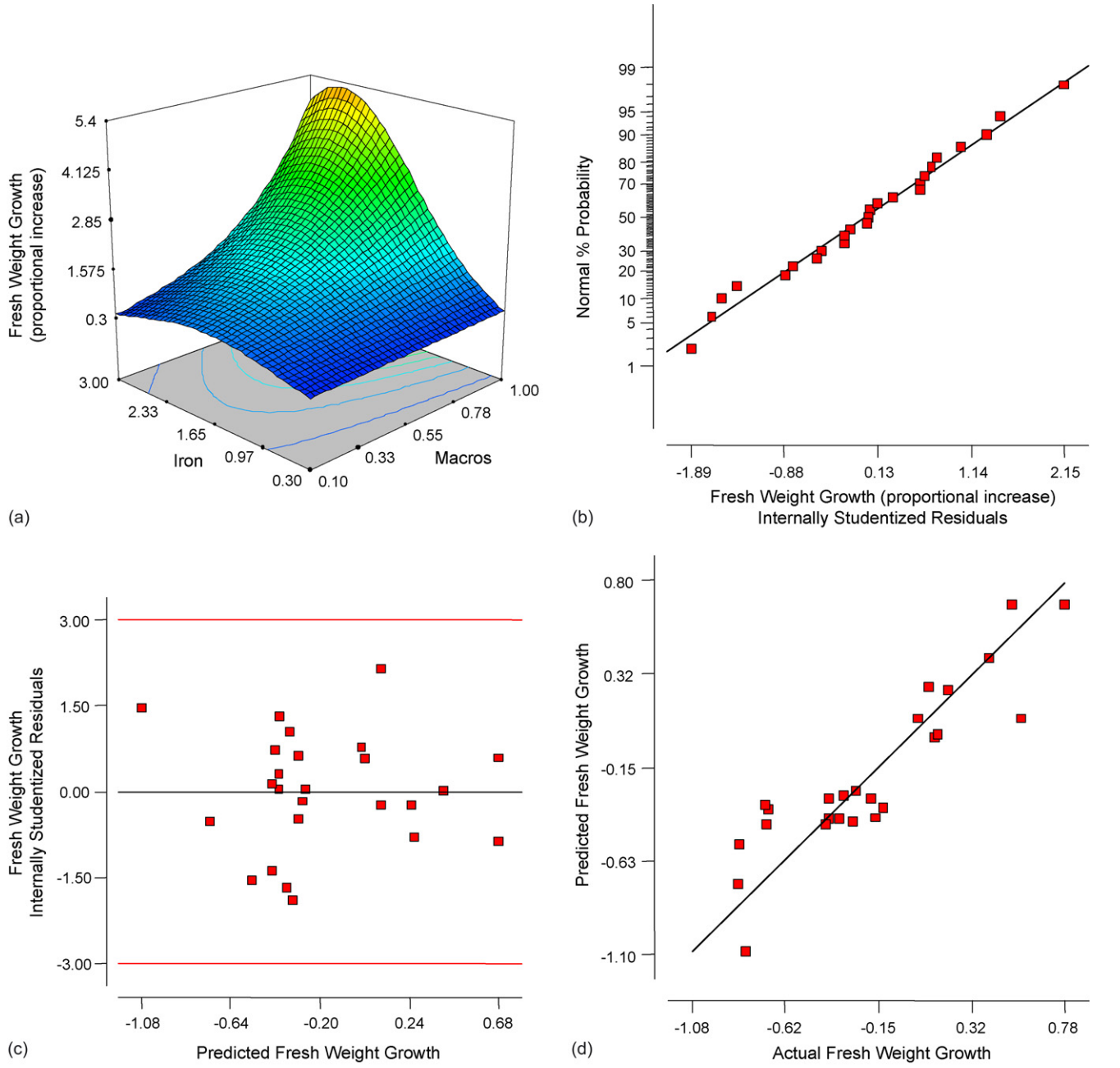

Fig. 4. Effect of macros and iron levels on sweet orange callus fresh weight accumulation including diagnostic plots of model adequacy. (a) Proportional fresh weight accumulation vs. iron and macros. Micros held constant at 3×. The quadratic polynomial equation plotted is $\log_{10}$ proportional fresh weight accumulation = −0.50615 + 0.030446*[Macro] − 0.042587*[Micro] + 0.36557*[Iron] + 0.39063*[Macro]*[Iron] + 0.097603*[Micro]*[Iron] − 0.20755*[Iron]^2. The actual values, rather than the transformed values, are plotted for clarity. (b) Normal plot of residuals for internally studentized residuals of $\log_{10}$ fresh weight accumulation data. (c) Residuals vs. predicted $\log_{10}$ fresh weight accumulation plot. (d) Predicted vs. actual $\log_{10}$ fresh weight accumulation plot.

selecting two treatments not included in the original design, measuring the fresh weight accumulation of those treatments, and comparing the responses to the predictions. The treatments included two points in the response volume (i.e., there are three dimensions in the design space), not tested in the original experiment. The first point was where the fresh weight accumulation was predicted to be the highest, or 1× Macros–3× Micros–2.53× Iron. The second point in the response volume was MS basal salt levels, 1× Macros–1× Micros–1× Iron – the salt levels we currently use to maintain this callus culture. The predicted fresh weight accumulation for both treatments was 5.27× for the highest point and 1.34× for MS salts or a predicted 3.83× increase over MS basal salt levels. Running these treatments and comparing the resulting fresh weight accumulation between the predicted highest point and MS using a *t*-test resulted in a highly significant increase ($p = 0.0002$); in growth terms the fresh weight accumulation was increased 101%.

Table 3
ANOVA for sweet orange callus response variables fresh weight accumulation and the gestalt

Term	Fresh weight accumulation[a]	Gestalt[b]
Overall model—*F*-value (p-Value)[*]	13.75 (<0.0001)	19.82 (<0.0001)
Lack-of-fit—*F*-value (p-Value)[**]	1.14 (0.4790)	0.80 (0.6602)
R^2	0.8128	0.8685
Adjusted R^2	0.7536	0.8247
Predicted R^2	0.6488	0.7534
Adequate precision[***]	14.926	13.806

[*] The *F*-value for the overall model and the probability of a larger *F*-value. A $p < 0.05$ indicates a significant effect on the response measured.
[**] A $p > 0.05$ indicates no additional variation that might be accounted for using a better model.
[***] Design-Expert recommends a value greater than 4 to ensure adequate predictions.
[a] The *F*-value (p-value) of the individual fresh weight accumulation quadratic model terms were Macros − 32.72 (<0.0001); Micros − 8.34 (0.0094); Iron − 1.98 (0.1755); (Macros)(Iron) − 16.37 (0.0007); (Micros)(Iron) − 8.50 (0.0089); $(\text{Iron})^2$ − 13.73 (0.0015).
[b] The *F*-value (p-value) of the individual gestalt quadratic model terms Macros − 64.05 (<0.0001); Micros − 2.83 (0.1101); Iron − 10.82 (0.0041); (Macros)(Iron) − 14.83 (0.0015); $(\text{Micros})^2$ − 4.14 (0.0570); $(\text{Iron})^2$ − 16.01 (0.0008).

3.5. Sweet orange callus growth—gestalt

The gestalt response is shown in Fig. 5a. The gestalt response was an overall assessment of the ''quality'' of the culture and was scored using the simple 1–2–3 system described above. As with the *Brugmansia* scoring was easy and rapid. The normality assumption was examined (Fig. 5b) and, although there is some slight curvature around the line the normality assumption appears reasonable. The variance scatter does not reveal any obvious distortions that would indicate a violation of the assumption (Fig. 5c); the line pattern of observations is simply a function of the gestalt ranging from 1 to 3 (note that these are the transformed gestalt values so the line pattern are the treatments with a gestalt of one). The outlier *t*-test and Box–Cox plot (not shown) did not indicate any outliers or a need for data transformation. The lack-of-fit was not significant ($p = 0.6602$). The predicted gestalt versus the actual gestalt values (Fig. 5d) indicated that the model is useful for prediction as the points are evenly above and below the 1:1 regression line.

A summary of the ANOVA, R^2, and adequate precision statistics for the gestalt response is presented in Table 3. The quadratic model is highly significant. The lack-of-fit and adequate precision statistics are acceptable and indicate that the model is suitable for predicting this response. Since the maximum value of the gestalt could not exceed three it is not useful to think of a maximum high point such as we did for fresh weight accumulation. Fig. 5a does show a region of the response surface that exceeds three, but this is simply a characteristic of the fitted polynomial. However, a desirability function (Myers and Montgomery, 2002) can be used to define any region of the response surface where the gestalt is equal to, greater than, or less than some value or combination of values relevant to the experimental objectives. For example, specifying that any predicted gestalt value ≥ 3 will be assigned a desirability value of 1, and gestalt values less than 3 corresponding lower desirability values results in the plot in Fig. 5e. Notice the large flat plateau region with a desirability of 1—this is where the predicted gestalt is ≥ 3. This means that any treatment combination on the plateau region will result in callus that is of high ''quality.'' It should be pointed out for accuracy that the region is not really a plateau since iron was set at 1.65× for Fig. 5e. The desirability plateau is actually a volume function and the plateau represents a ''slice'' through that volume (i.e., we cannot simultaneously view all three response dimensions—macros, minors, and iron). The predicted R^2-value for the gestalt response is 0.8927 and indicates that the model is suitable for predicting new observations.

4. Discussion

To determine if overall quality could be precisely quantified using a gestalt assessment we chose two different in vitro systems, designed a single and triple factor RSM design, included both standard measurement-type response variables and a gestalt of culture quality response variable, and analyzed the data and the underlying statistical assumptions. For each experiment the gestalt modeled as precisely as the measured response variables while satisfying the statistical assumptions necessary for a valid analysis. Our results indicate that the gestalt response is a valid response as it was indistinguishable, in terms of statistical quality, from the measured responses. The gestalt evaluations in this study were conducted by one person for the *Brugmansia* experiment and one person for the sweet orange callus experiment. The results from multiple individual assessments could be averaged if the objective was more individually subjective such as occurs with sensory evaluation testing of foods.

One major difference between the gestalt and the measured responses was the speed of data collection. Scoring a culture basically involved the time it took to observe it and write down the assessment. All of the measured responses required substantially more time and effort to measure. Because of the ease of recording a gestalt measure it would add little to an experiment to include along with measured responses. When resources are limited or unavailable for measured data collection the gestalt may provide enough information to meet the experiment objectives. This is particularly true of commercial operations where quality, however defined, should be well known and readily recognized by the operators.

One conceptual issue that a gestalt analysis highlights is the difference between maximal and optimal. For example, the fresh weight accumulation of the sweet orange callus could be substantially increased over MS salt formulation used to maintain the culture. However, it may not be desirable to use a formulation where the callus grows rapidly if the objective is to develop a maintenance medium, as the callus would require more frequent transfers. Therefore, an optimal maintenance

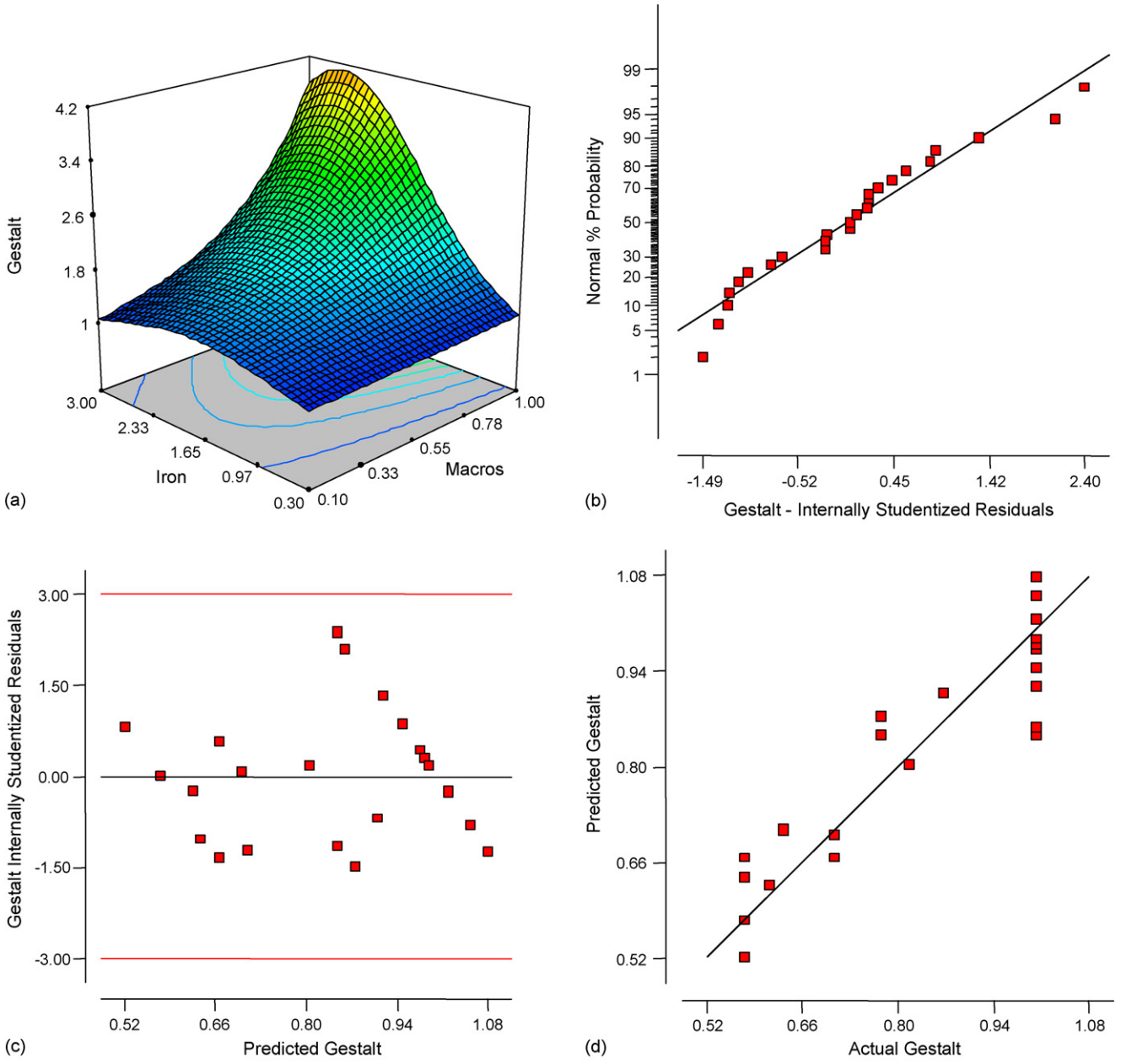

Fig. 5. Effect of macros and iron levels on the sweet orange callus gestalt including diagnostic plots of model adequacy and desirability. Note that all gestalt values were transformed by the inverse square root. (a) Gestalt vs. macros and iron. Micros held at 1.65×. The polynomial equation plotted is 1.0/Sqrt(gestalt) = 1.17452 − 0.12005*[Macro] − 0.15554*[Micro] − 0.24335*[Iron] − 0.12247*[Macro]*[Iron] + 0.040118*[Micro]^2 + 0.080562*[Iron]^2. The actual values, rather than the transformed values, are plotted for clarity. (b) Normal plot of residuals for internally studentized residual 1.0/Sqrt gestalt data. (c) Residuals vs. predicted 1.0/Sqrt gestalt plot. (d) Predicted vs. actual 1.0/Sqrt gestalt plot. (e) Gestalt desirability vs. iron and macros plot. Micros held at 1.77×.

medium might be a formulation where the callus grows more slowly but is still a ‘‘high quality’’ callus. This is a problem of multiple response optimization, a particularly useful application of response surface methodology. The approach would be to minimize the fresh weight accumulation and maximize the gestalt; this could be done mathematically or visually via contour plot overlays. Furthermore, the idea of an optimal response implies a gestalt. For example, how would the question be answered, ‘‘What is the optimal formulation for fresh weight accumulation?’’ Without an objective or context there is no answer to this question. The objective to grow callus as rapidly as possible provides one answer; a formulation to grow callus more slowly for general maintenance provides another. However, the experimenter knows that there are formulations where the callus grows slowly because it is growing poorly. How can these formulations be distinguished from those where the callus grows slowly but remains ‘‘healthy?’’ Additional measured response variables could be added to try and capture callus health (e.g., friability, color, phenolic types, and levels, etc.). Or, a simple gestalt could be performed and relevant formulations quickly identified.

Subjective rating systems are commonly used in plant biology research (Altman and Goren, 1974; Ben-Hayyim and Goffer, 1989; Castillo and Jordan, 1997; Chaleff and Parsons,

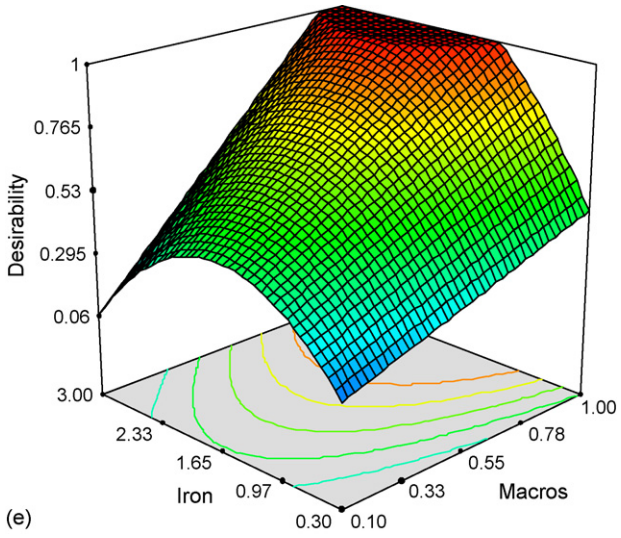

Fig. 5. (*Continued*).

1978; Chaturvedi and Mitra, 1975; Chen et al., 1987; Ge et al., 2006; Gleddie et al., 1986; Hanhineva et al., 2005; Hiratsuka and Katagiri, 1988; Jia, 1982; Jiang et al., 2005; Kao and Michayluk, 1975; Kerns et al., 1986; Khunachak et al., 1987; Kochba et al., 1978; Kong and Chin, 1988; Maene and Debergh, 1985; Matsuoka and Hinata, 1979; Mori et al., 2005; Niedz et al., 1985; Nito and Iwamasa, 1990; Shahin, 1985; Sudha et al., 2005; Uddin et al., 2005; van der Fits et al., 2000). The primary reason rating scales are used, including gestalt analysis, is that they generally provide important information about a response, but are usually simpler, faster, and more economical than measured responses. Rating systems for in vitro responses typically involve scales of 3–5 categories such as "−" to "++++" where a "−" score signifies no response and the number of +'s (typically 2–4) signifies an increasing response. Second, rating scales are commonly used to estimate a single quantitative measure. For example, rather then weighing callus to obtain milligram or gram weights for each treatment, a tedious procedure, researchers commonly use a rating scale to rapidly score the "degree of callusing" where a "−" signifies no callus and the number of +'s signifies the extent of callusing. The gestalt response is not a proxy for a single quantitative measure, but rather a measure of overall quality. The gestalt response is inherently multivariate since overall quality is composed of multiple responses that may not all be known or identifiable. For example, an experienced tissue culturist could use a gestalt scale that would simultaneously include all the definable and undefinable aspects of overall callus quality (e.g., growth, color, friability, odor, number of embryos/shoots produced, etc.). Third, ranking systems are considered less reliable than the quantitative measures they estimate. We were unable to find a single study in the plant tissue culture literature where the ranking scores were statistically analyzed. This contrasts to the standard analyses that generally accompany quantitative measures/data. Our results suggest that ranking data for in vitro responses can be as useful as quantitatively measured variables if the ranking system is applied with discipline and is subject to a rigorous statistical analysis such as presented here.

Would a gestalt analysis be useful in other areas of horticultural research? An underlying and defining aspect of all horticulture is that the plants contribute a beneficial quality to our lives that affects all of our senses. The beauty of the grass, flowers, shrubs, and trees that comprise a garden landscape, or the fruits, vegetables, nuts, and spices that comprise an attractive and flavorful meal both exhibit a strong gestalt where the whole is perceived as greater than its individual parts. Tredici (2002) recognized that there is an overall quality aspect to tree growth and morphology that cannot be totally accounted for with any single metric. Admittedly, the two tissue culture examples illustrated in this study are simple, but since the gestalt modeled well it may be worth the effort to examine its general use in horticultural science. There are at least two benefits in identifying horticultural processes and products that can be analyzed by a gestalt measurement. First, the inherent speed and accuracy of the measurement make it immediately applicable. Second, by correlation studies between measured responses the gestalt can be used to identify the primary component variables of overall quality; new processes or products may then be possible to the extent that these components can be manipulated or altered.

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